Microscopy

For imaging of samples a Leica TCS SP5 laser scanning microscope mounted on a Leica DMI 6000 CS inverted microscope (Leica Microsystems, Germany) with an HCX plan apo 40× or 63× oil immersion objective was used. For image analysis we used ImageJ (Image Processing and Analysis in Java) software (http://rsbweb.nih.gov/ij/).

Localization

- Cells were seeded onto 8-well microscope slides (Ibidi) and after 24 h transfected with selected plasmids.
- After 24 h after transfection cells were fixed, stained with corresponding primary antibodies (anti-AU1, HA, Myc or Flag tag), washed and stained with fluorescent secondary antibodies.
- Successive images were acquired at appropriate excitation and emission settings.

Calcium imaging

Fluorescent indicators Fura Red, AM (Setareh Biotech LLC) and Fluo-4, AM (Biotium) were used for ratiometric measurement of Ca^{2+} influx into the cells. For video analysis we used CaPTURE software, which was developed by our team.

- Cells were seeded on a 6-well glass-bottom plate (Cellvis) and after 24 h transfected with selected plasmids.
- 24 h after transfection cells were loaded with fluorescent calcium indicators

Loading with fluorescent calcium indicators

- Fluo-4, AM and Fura Red, AM from 5 mM stock solutions in DMSO were mixed in a 1:3.75 molar ratio and immediately before use 20% (w/v) Pluronic F-127 was added in a molar ratio 1:1.
- Mixture of calcium indicators and Pluronic F-127 was then diluted in DMEM, supplemented with 10% heat-inactivated FBS.
- Medium with calcium indicators was added to the wells and cells were incubated for 30 min at 37°C and 5% CO2.
- Medium was discarded and the cells were washed with 1x PBS.
- Fresh DMEM with 10% heat-inactivated FBS and 4 mM CaCl2 was added 10 min before starting the stimulation.